(2) H-Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His-OH (see, for example, Claims 11, 15 and 27);

(3) Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His (see, for example, Claims 2 and 16);

(4) His Pro Leu Gly Ser Pro Gly Ser Ala Ser Asp Leu Glu Thr Ser Gly Leu Gln Glu Gln Arg Asn His Leu Gln Gly Lys Leu Ser Glu Leu Gln Val Glu Gln Thr Ser Leu Glu Pro Leu Gln Glu Ser Pro Arg Pro Thr Gly Val Trp Lys Ser Arg Glu Val Ala Thr Glu Gly Ile Arg Gly His Arg Lys Met Val Leu Tyr Thr Leu Arg Ala Pro Arg Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu (see, for example, Claims 3 and 17); and

(5) Met Asp Pro Gln Thr Ala Pro Ser Arg Ala Leu Leu Leu Leu Phe Leu His Leu Ala Phe Leu Gly Gly Arg Ser His Pro Leu Gly Ser Pro Gly Ser Ala Ser Asp Leu Glu Thr Ser Gly Leu Gln Glu Gln Arg Asn His Leu Gln Gly Lys Leu Ser Glu Leu Gln Val Glu Gln Thr Ser Leu Glu Pro Leu Gln Glu Ser Pro Arg Pro Thr Gly Val Trp Lys Ser Arg Glu Val Ala Thr Glu Gly Ile Arg Gly His Arg Lys Met Val Leu Tyr Thr Leu Arg Ala Pro Arg Ser Pro Lys Met Val Gln Gly Ser Gly Cys Phe Gly Arg Lys Met Asp Arg Ile Ser Ser Ser Ser Gly Leu Gly Cys Lys Val Leu Arg Arg His (see, for example, Claims 4 and 18).

The present invention also concerns a recombinant DNA sequence comprising a base sequence encoding a polypeptide having one of the amino acid sequences (1)-(5) above, as well as a method of producing cDNA, comprising:

hybridizing a probe having a DNA sequence encoding a part of porcine brain natriuretic peptide to a human cDNA library;

selecting a positive clone; and

isolating said cDNA of said positive clone.

As demonstrated by the factual evidence and statements in the executed Declaration of Dr. Tetsuji Sudoh dated October 12, 1994, and filed October 20, 1994 (a copy of which is attached hereto for convenience), one of ordinary skill would not have had a reasonable expectation of success in arriving at the present invention from the knowledge present in the art at the time grandparent application Serial No. 07/486,827 was filed (March 1, 1990).

For example, neither the 70% degree of homology between human atrial natriuretic peptide (hANP) and porcine BNP (pBNP) taught by Sudoh et al (*Biochem. Biophys. Res. Comm.*, 155:726-732 and *Nature*, 332:78-80, each of which is cited against the present application) nor the 50.6-65.7% degree of homology between hANP mRNA and pBNP mRNA taught by Maekawa et al (also cited against the present application) is sufficiently high for one of ordinary skill to reasonably expect success in cloning and isolating the cDNA of one based on the sequence of the other (see paragraph 5, page 3 of the Declaration).

Further, Table 1 of Oikawa et al (also cited against the present application) teaches that the homology between hANP and other mammalian ANPs is only 52-60%. Thus, assuming that one of ordinary skill expects the same degree of homology between hBNP and other mammalian BNPs as is observed between hANP and other mammalian ANPs, Sudoh et al (*Biochem. Biophys. Res. Comm.*, 155:726-732 and *Nature*, 332:78-80), Maekawa et al and Oikawa et al appear to indicate that the degree of homology is greater between pBNP and hANP than what one expects between pBNP and hBNP. As a result, one might expect a probe based on the pBNP gene to lead to cloning of a hANP gene, rather than a hBNP gene (see paragraph 6, pages 3-4 of the Declaration).

In addition, Sudoh et al (*Biochem. Biophys. Res. Comm.*, 159:1427-1434, submitted with and incorporated into the Declaration by reference) disclose that human and porcine ANP's have 89.7% and 100% identical residues in the pro-form and α-form, respectively (page 1433, lines 1-3). However, the high homology between the pro- and α-forms of hANP and pANP would lead one to reasonable expect success in cloning and isolating hBNP cDNA using a 10-20 bp pBNP probe, which the present Inventors attempted to do, but failed to successfully carry out (see paragraph 7, page 4 of the Declaration).

Furthermore, the low homology (70.0%) between human prepro-BNP and porcine prepro-BNP (results determined by the present Inventors, disclosed by Sudoh et al [*Biochem. Biophys. Res. Comm.*, 159:1427-1434]) presents a sharp contrast to the

more highly conserved mammalian ANP's, thus introducing an unexpected problem in cloning hBNP. This unexpected problem makes it surprising that hBNP cDNA could be cloned and isolated, given the level of ordinary skill and the knowledge in the art at the time of filing grandparent U.S. application Serial No. 07/486,827 (March 1, 1990) (see paragraph 9, page 4 of the Declaration).

Therefore, one of ordinary skill would not have had a reasonable expectation of success in arriving at the present invention from the knowledge present in the art at the time grandparent application Serial No. 07/486,827 was filed.

Furthermore, as discussed in the Amendment filed July 21, 1993 and the Preliminary Amendment filed February 7, 1994, Seilhamer et al, U.S. Patent No. 5,114,923, disclose that the cDNA encoding porcine BNP completely failed to hybridize to human DNA (see col. 8, lines 40-54 and col. 9, lines 32-35 and 45-47 therein). However, Seilhamer et al successfully used the pBNP cDNA to retrieve genes encoding related proteins from other species, such as a pig, a rat, a dog, a cat and a rabbit (col. 9, lines 32-44).

Therefore, the Examiner's assertion that the appropriate probe to use would have been the complete genomic pBNP gene may not be entirely accurate, since Seilhamer et al successfully used the pBNP cDNA to retrieve the corresponding genes from five out of six species attempted. Further, the Examiner's assertion that it is not surprising that Seilhamer et al fail to isolate the human BNP genomic clone appears to

be a hindsight reconstruction, rather than an accurate interpretation of the teachings of Seilhamer et al. Seilhamer et al specifically disclose:

> "There was every reason to believe that porcine BNP should be capable of hybridizing to human NRP under appropriate conditions; however no conditions could be determined under which pBNP would hybridize to a human library. This was surprising in that, in an evolutionary sense, porcine BNP is more likely to be related to the corresponding human protein, than is a corresponding canine protein. It was thus unexpected that porcine BNP was unable to identify human NRP...." (col. 8, lines 44-54; emphasis added).

Consequently, the Examiner's conclusion that it is not surprising that Seilhamer et al fail to isolate the human BNP genomic clone using pBNP cDNA is not accurate. As a result, in view of the failure of Seilhamer et al to hybridize DNA encoding pBNP to human DNA, the success of the present Inventors in obtaining DNA encoding human BNP is surprising and unexpected. Thus, the present cDNA, recombinant DNA sequence and method are not obvious, based on the evidence of record.

Furthermore, the structural differences between the present DNA and the closest DNA of the cited references are not obvious. For example, Maekawa et al, Sudoh et al (R) and Sudoh et al (T) each disclose a form of porcine BNP (see, for example, page 412, Figure 1 of Maekawa et al). The formulas of porcine BNP-32 is compared to the three smallest formulas of the present cDNA and recombinant DNA sequences hereinbelow:

	1												13
pBNP-32:	Ser	Pro	Lys	**Thr**	**Met**	**Arg**	**Asp**	Ser	Gly	Cys	Phe	Gly	Arg
hBNP-32[1]:	Ser	Pro	Lys	**Met**	**Val**	**Gln**	**Gly**	Ser	Gly	Cys	Phe	Gly	Arg
hBNP-26[2]:							**Gly**	Ser	Gly	Cys	Phe	Gly	Arg
hBNP-23[3]:										Cys	Phe	Gly	Arg

	14												26
pBNP-32:	**Arg**	**Leu**	Asp	Arg	Ile	**Gly**	Ser	**Leu**	Ser	Gly	Leu	Gly	Cys
hBNP-32[1]:	**Lys**	**Met**	Asp	Arg	Ile	**Ser**	Ser	**Ser**	Ser	Gly	Leu	Gly	Cys
hBNP-26[2]:	**Lys**	**Met**	Asp	Arg	Ile	**Ser**	Ser	**Ser**	Ser	Gly	Leu	Gly	Cys
hBNP-23[3]:	**Lys**	**Met**	Asp	Arg	Ile	**Ser**	Ser	**Ser**	Ser	Gly	Leu	Gly	Cys

	27					32
pBNP-32:	**Asn**	Val	Leu	Arg	Arg	**Tyr**
hBNP-32[1]:	**Lys**	Val	Leu	Arg	Arg	**His**
hBNP-26[2]:	**Lys**	Val	Leu	Arg	Arg	**His**
hBNP-23[3]:	**Lys**	Val	Leu	Arg	Arg	**His**

[1]: formula (3) on p. 2 above; see claims 2 and 16
[2]: formula (2) on p. 2 above; see claims 11, 15 and 27
[3]: formula (1) on p. 1 above; see claims 10 and 14

A number of differences exist in the amino acid sequence of pBNP-32 and the amino acid sequences encoded by the present polynucleic acids. A comparison of the codons which encode the different amino acid residues provides an indication of the minimum number of differences in the corresponding polynucleic acids. A summary of the differences is presented in the following table, in which "N" refers to any nucleotide:

Position	Present Invention	Codons*	pBNP-32	Codons*
32	His	CAT CAC	Tyr	TAT TAC
27	Lys	AAA AAG	Asn	AAT AAC
21	Ser	AGT AGC TCN	Leu	TTA TTG CTC CTA CTG
19	Ser	AGT AGC TCN	Gly	GGN
15	Met	ATG	Leu	TTA TTG CTC CTA CTG
14	Lys	AAA AAG	Arg	AGA AGG CGN
7	Gly	GGN	Asp	GAT GAC
6	Gln	CAA CAG	Arg	AGA AGG CGN
5	Val	GTN	Met	ATG
4	Met	ATG	Thr	ACN

*: The identities of the codons which encode each of the above amino acids was obtained from Dressler et al, "Discovering Enzymes," Scientific American Library Series, p. 186, (1991), submitted with the Amendment and Request for Reconsideration filed December 30, 1991.

As shown by the above table, for each difference in amino acid sequence, at least one difference in DNA sequence exists.

Consequently, the structure of the smallest cDNA and recombinant DNA sequence of the present invention (the sequence of formula (1) above) contain at least 6 differences from the structurally closest possible polynucleotide of the cited references (i.e., encoding the same number of amino acids in the same relative location in the gene). By a similar analysis, the structure of the present cDNA and recombinant DNA sequence of formula (2) above (Claims 11, 15 and 27) contain at least 7 differences from the structurally closest possible polynucleotide of the cited references, and the structure of the present cDNA and recombinant DNA sequence of formula (3) above (Claims 2 and 16) contain at least 10 differences from the structurally closest possible polynucleotide of the cited references. As one can imagine, the longer the length of the sequence being claimed, the greater the number of structural differences.

As was explained (in part) above and as will be explained (in part) below, the cited references do not suggest that the differences in structure between (1) the present cDNA and recombinant DNA sequence and (2) the structurally closest possible polynucleotide of the cited references is desirable, or would even result in a DNA which encodes a biologically active polypeptide. Consequently, one would not be motivated to make the structural changes to the structurally closest possible polynucleotide of the cited references in order to arrive at the present cDNA and recombinant DNA sequence.

Even further, the present invention provides a means for obtaining a human natriuretic peptide (hBNP) which is expected to have considerably higher rectum relaxation activity than the corresponding hANP, based on the known 3- to 4-fold increase in the rectum relaxation activity of pBNP relative to pANP (see page 2, lines 3-15 of the present specification). Consequently, the present invention represents a significant and meaningful advancement in this field.

The evidence of record clearly establishes that no reasonable expectation of success existed in arriving at the present invention (both DNA and method), based on the teachings and disclosures of the cited references. Further, the present cDNA and recombinant DNA sequences are structurally non-obvious from the closest possible polynucleotide suggested by the cited references. Therefore, the present invention is fully patentable over the cited references.

The rejection of Claims 2-7 and 10-23 under 35 U.S.C. 103 as being unpatentable over Maekawa et al in view of Maniatis, Sudoh et al, (R) and Sudoh et al (T), Oikawa et al and Vlasuk et al is respectfully traversed.

Maekawa et al disclose cloning and sequence analysis of cDNA encoding a precursor for porcine brain natriuretic peptide (pBNP). Sudoh et al (R) disclose the sequences of porcine BNP. Sudoh et al (T) disclose a 32-amino acid-long brain natriuretic peptide identified in porcine brain. Oikawa et al disclose the structure of dog and rabbit precursors of

atrial natriuretic peptides deduced from nucleotide sequences of cloned cDNA. Vlasuk et al disclose the structure and analysis of the bovine atrial natriuretic peptide precursor gene.

On page 228, Maniatis et al teach a method for identifying cDNA clones corresponding to developmentally regulated mRNAs. A population of cDNA molecules enriched in sequences characteristic for a particular developmental stage is used to probe a cDNA or genomic library. Given this equivalence of a cDNA and a genomic library, it is not clear how the teachings of Maniatis et al on page 228 cure the inability of Seilhamer et al to probe human DNA with porcine cDNA. Maniatis et al suggest that the same result is obtainable using either a cDNA or a genomic library. Thus, if one cannot achieve success with one library, Maniatis et al would lead not lead one to expect success using the other library.

Furthermore, the procedure of Maniatis et al appears to require that a probe from the same cell (i.e., the same tissue and the same species) be used. For example, on page 227, Maniatis et al teach that differential hybridization (the technique relied upon by the Examiner) is used when mRNA preparations are available which contain many sequences in common, except for the presence or absence of a few species of interest. Differential hybridization is taught as being useful for identifying certain mRNAs which may be present in a particular sample in which production of the mRNA has been

induced (e.g., by heat shock, drugs, hormones or a particular substrate). This strongly suggests that the genetic material for each of the mRNA preparations comes from the same source, but is treated differently in one of the preparations so as to induce the formation of a particular mRNA in that preparation.

Maniatis et al neither teach nor suggest that successful examples employed cDNA from another species to identify the inducible genes from a second species. Thus, the technique of differential hybridization appears to be relevant to obtaining a clone from the same species, based on the teachings of Maniatis et al immediately preceding the disclosure relied upon for the present rejection.

In the disclosure relied upon for the present rejection, Maniatis et al teach that cDNA prepared from mRNA obtained at one developmental stage (stage 1) is hybridized to a 20-fold excess of mRNA obtained from another stage (stage 2), and the hybrid is removed. This procedure of Maniatis et al is then repeated twice more using a 50-100-fold excess of stage 2 mRNA. The unbound cDNA fraction is then hybridized to a 100-fold excess of stage 1 mRNA, and the hybrid is recovered. After removing the mRNA, the cDNA that is highly enriched in stage 1-specific sequences is used as a probe.

One would not reasonably expect success using the procedure described by Maniatis et al on page 228 if a probe from a different species is used. If mRNA from a different organism is used, the polynucleotide homology differences between the two organisms may be sufficiently great as to

prevent hybridization, particularly since the two mRNA preparations are obtained from different developmental stages. A difference in the developmental stages induces differences in mRNAs, even when obtained from the same organism or cell. To minimize the differences between the two mRNAs and thus maximize the chances of success (note the prior teaching of Maniatis et al that the two mRNA preparations contain many sequences in common, but differ from each other based on the presence or absence of a few species of interest), it is apparent that Maniatis et al refer to mRNA obtained from different developmental stages of the same organism.

Based on the steps necessary to arrive at the present invention (hBNP) from the closest subject matter disclosed by the cited references (pBNP), one must use a probe from a different species. The rejection cannot be properly based on use of a human DNA probe, because (1) the cited references do not disclose hBNP DNA and (2) use of any other human gene would lead to isolation and/or identification of that other human gene.

Furthermore, in the present invention, a cDNA library was prepared from mRNA obtained from a human tissue. The human BNP clone was screened using a cDNA fragment encoding porcine BNP-26 and the 30 bp upstream therefrom as a probe (page 14, lines 1-12 of the present specification). Fifty-five positive plaques were obtained (page 15, lines 2-3). Thus, it appears that the present polynucleotides were not obtained by differential hybridization. Accordingly, the relevance of the

process of Maniatis et al identified by the Examiner as being most pertinent to the present invention is not understood.

Consequently, it is not seen how the teachings of Maniatis et al cure the deficiencies of the remaining cited references. The method of Maniatis et al appears to require that a probe from the same cell (i.e., the same tissue and the same species) be used. Further, there is no evidence that the method of Maniatis et al relied upon by the Examiner is reasonably likely to be successful in arriving at the present invention from the closest polynucleotides disclosed or suggested by the cited references. Consequently, the present invention is fully patentable over the cited references.

However, assuming *arguendo* that it would have been obvious for one of ordinary skill in the art to use the porcine DNA sequence disclosed by Maekawa et al, or an effective portion thereof (as taught by Seilhamer et al [col. 8, lines 40-54]), as a probe to screen for the human BNP gene, the factual bases by which one of ordinary skill evaluates the expectation of success (as set forth in the Declaration of Sudoh dated October 12, 1994, and filed October 20, 1994) establish that one of ordinary skill would not have a reasonable expectation of success in arriving at the present invention from the teachings of the cited references. Thus, the evidence of record supports the patentability of the present invention.

Furthermore, the clear failure of Seilhamer et al to successfully achieve the result obtained by the present

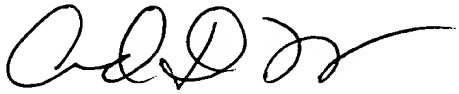
Inventors also attests to the nonobviousness of the present DNA. Interestingly, as discussed above, both Seilhamer et al and Maniatis et al equate a genomic library with a cDNA library, thereby indicating that no unusual problems are expected in screening a genomic library. Even more interesting, the temperature conditions used by Seilhamer et al were less stringent (37-42°C; col. 9, lines 6-10 and 38-42) than those used by the present Inventors to obtain the present DNA (60°C; page 14, line 19 of the present specification).

Therefore, the combined teachings of the cited references attest to the difficulties encountered in arriving at the present invention, as described (1) in the Declaration of Sudoh and (2) by Seilhamer et al. The cited references do not suggest that the modifications necessary to arrive at the present polynucleotides from the closest polynucleotide(s) disclosed in or suggested by the cited references are desirable, or would even result in a gene which encodes a functional polypeptide. Further, the cited references appear to lead one away from the presently claimed method (obtaining an hBNP DNA from pBNP DNA). Therefore, this ground of rejection is unsustainable, and should be withdrawn.

Accordingly, the present application is in condition for allowance. Early notice to that effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



Norman F. Oblon
Attorney of Record
Registration No. 24,618

Andrew D. Fortney, Ph.D.
Registration No. 34,600

Crystal Square Five - Fourth Floor
1755 Jefferson Davis Highway
Arlington, VA 22202
Phone: (703) 413-3000
Fax #: (703) 413-2220
ADF/smi

C

O.S.&M. File No. 1587-015-0 FWC I By NFO:ADF:dsf

Serial No. 08/192,800

In the Matter of the Application of TETSUJI SUDOH ET AL

For: PHYSIOLOGICALLY ACTIVE POLYPEPTIDE AND DNA

The following has been received in the U.S. Patent Office on the date stamped hereon:

[ ] _____ pps. Specification & _____ Claims/Drawings _____ Sheets
[ ] Combined Declaration, Petition & Power of Attorney (____ pages)
[ ] List of Inventor Names and Addresses
[ ] Rule 60 Application; [ ] Rule 62 Application
[ ] Notice of Priority; [ ] Priority Doc. ( )
[ ] Check for $_____; XXX Dep. Acct. Order Form
[ ] Assignment _____ pages/PTO-1595
[ ] Letter to Official Draftsman
[ ] Letter Requesting Approval of Drawing Changes
[ ] Drawings ____ sheets
XXX Letter
[ ] Amendment
[ ] Information Disclosure Statement; [ ] PTO-1449
[ ] Cited References ( )
[ ] _____ Search Report
[ ] Statement of Relevancy
[ ] IDS/Related/List of Related Cases
[ ] Restriction Response [ ] Election Response
XXX Rule 132 Declaration (executed)
[ ] Petition
[ ] Notice of Appeal
[ ] Brief
[ ] Issue Fee Transmittal
[ ]
[ ]
[ ]

MAIL ROOM OCT 20 1994 PAT. & TRADEMARK OFF.

Rev. 9/94